REMARKS

Applicants respectfully request favorable reconsideration of this application, as amended. As discussed below, Claims 1–3 and 9–11 are pending.

Applicants note with appreciation the recognition of the claims for domestic and foreign priority.

The Oath was objected to as not conforming to 37 C.F.R. § 1.69. *See*, Office Action at Page 2. In response, an English Declaration has been submitted herewith. Applicants respectfully submit that the objection to the Oath has been overcome.

The Office Action noted that "no IDS was filed with this application" (Page 2). Applicants thank the examiner for this reminder, and an Information Disclosure Statement has been submitted herewith. The references cited therein were identified in the parent application's International Search Report mailed on April 8, 2004.

The Specification was objected to for containing typographical error and grammatical problems. The Applicant has reviewed the Specification with particularity, and a Substitute Specification (marked-up and clean versions) has been submitted herewith that incorporates responsive amendments. No new matter has been added, and Applicants respectfully submit that the objection to the Specification has been overcome.

Claims 1–3 were rejected under 35 U.S.C. § 102(b) as being anticipated by Okura (STN abstract of WO/1995/06032). Claims 4, 6 and 7 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Afifi (Abstract of Revue Roumaine de Chimie (1983), 28(8), 849-55) in view of March (March's Advanced Organic Chemistry, 5th ed., (2001) Wiley, 2083 pages), Claims 5 and 8 were rejected as being unpatentable over Okura in view of March and Greene (Protective Groups in Organic Synthesis, Greene, 3rd ed., New York John Wiley & Sons, Inc., 1999, 779 pages), and Claim 9 was rejected as being unpatentable over Okura in view of Neye (Abstract of Exp Clin Endocrinol Diabetes; 1998; 106(4):292-8).

In the interests of securing an expedited Notice of Allowance, and without acceding to the rejections, Claims 1–3 have been amended to recite certain features of the claimed invention more perspicuously, as discussed below. Claims 4–8 have been canceled without prejudice, and Applicants reserve the right to pursue this subject matter in one or more continuation applications. Claims 10 and 11, depending from Claim 1, have been added. Support for these amendments may be found, for example, in the Specification at Paragraphs 0040 to 0067, etc. No new matter has been added, and Applicants respectfully

submit that none of the cited references, taken either singly or in combination, teaches or suggests these features.

Claim 1 is Allowable Over Okura

Applicants respectfully submit that the compounds of the present invention are novel non-peptide glucagon-like peptide-1 receptor (GLP-1R) agonists, and that these compounds can be used to prepare the medicaments for treating carbohydrate metabolism disturbance-related diseases such as type II diabetes, insensitivity to insulin or obesity, etc. In the interests of receiving an expedited Notice of Allowance, and without acceding to the rejections, Claims 1–3 have been amended to exclude the compounds disclosed by Okura. Consequently, Applicants respectfully submit that Okura fails to disclose (or even suggest) all of the features recited by Claim 1, as well as Claims 2 and 3.

Accordingly, Applicants submit that Claim 1 is allowable over the cited references. Furthermore, Claims 2, 3, and 9–11, depending from Claim 1, are also allowable, at least for the reasons discussed above. Applicants also submit that the cited references fail to teach or suggest many of the features recited by the dependent claims, and, consequently, that these claims are independently allowable.

In view of the foregoing amendments and remarks, Applicants respectfully submit that this application is in condition for allowance and should now be passed to issue. A Notice of Allowance is respectfully solicited.

If any extension of time is required in connection with the filing of this paper and has not been requested separately, such extension is hereby requested. The Commissioner is hereby authorized to charge any fees and to credit any overpayments that may be required by this paper under 37 C.F.R. §§ 1.16 and 1.17 to Deposit Account No. 50-2036.

Respectfully submitted,

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June 15, 2009 By: _____

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#102787523_1

THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS, THE PREPARATION AND THE USE OF THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a National Stage of Patent Cooperation Treaty (PCT) Application No. PCT/CN2003/001115, filed December 25, 2003, entitled, THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS, THE PREPARATION AND THE USE OF THE SAME, which claims priority to Chinese Patent Application No. 200310109331.0, filed December 12, 2003, all of the disclosure of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a group of glucagon-like peptide-1 receptor (GLP-1R) agonists. In particularly, the present invention relates to a group of small molecular organic compounds of substituted five-membered heterocyclic ring derivatives which may be used as non-peptide GLP-1R agonists. The compounds of the present invention may be used as medicaments for treating the glycometabolism disturbance-related diseases such as type II diabetes, insensitivity to insulin and obesity etc. And, the present invention also relates to a process for manufacturing the said GLP-1R agonists.

BACKGROUND OF THE INVENTION

[0003] Diabetes mellitus (DM) is a common endocrine metabolic disease with heredity tendency. It is caused mainly by the absolute or relative hyposecretion of the insulin, and it causes metabolic disturbance of saccharide, fat, protein, and subsequently vitamin, water and electrolyte. The manifestations include the increase of glycemia and urine glucose, and the patients have the symptoms of polydipsia, polyphagia, polyuria, dry mouth, and general weakness. The morbidity rate of diabetes mellitus is 1 to 5%, and shows a trend of gradually increasing. Diabetes

mellitus, cancer, and cardiovascular diseases are referred as three worldwide serious diseases. The object of treating diabetes mellitus is to correct the disturbance of carbohydrate metabolism, so as to eliminate the symptoms, promote restoration of the function of pancreatic islet, improve the insulin resistance, maintain the better healthy condition and physical strength, and prevent and treat various complications.

[0004] Diabetes mellitus is commonly divided into two types: Insulin Dependent Diabetes Mellitus (type I, IDDM) and Non-Insulin Dependent Diabetes Mellitus (type II, NIDDM). Since the pathogenesis for theses two types of diabetes mellitus are different, the medicaments for treating them are far different, which are stated respectively as follows.

[0005] Type I diabetes mellitus is caused by virus infection in hereditarily susceptible person which ~~produce~~ produces the paradoxical reaction of the islet cells through ~~immunomechanism~~ immunoreactions, so that the pancreatic islets begin to be damaged and even ~~lost~~ lose their function completely. About 5% of diabetes mellitus is type I. At present, the medicaments for treating type I diabetes mellitus mainly include exogenous insulin (including human insulin and animal insulin), drugs having the insulin-like effect, insulin-like growth factor-1 (IGF-1), novel long-acting insulin preparation, and Jin Qi hypoglycemic tablet, etc.

[0006] ~~A few of type~~ Type II diabetes mellitus is caused by direct impair of β-islet cells which ~~decreases~~ decrease the secretion of insulin. ~~And most~~ Most of type II diabetes mellitus is caused by a combination of factors that may include genetic traits, life style, environmental contributors, metabolic disorders, obesity, and so on. In this disease state, muscular, hepatic and adipose tissues are insensitive to the insulin, which thereby ~~decreasing~~ decrease the intake of the glucose. Most of diabetics suffer from type II diabetes mellitus. At present, the medicaments for the clinical treatment of NIDDM mainly include sulphonylureases, biguanides, other hypoglycemic drugs and adjuvants, etc.

[0007] The sulphonylureas hypoglycemic drugs bind to the receptors on the cell membrane of β-islet cells to close the potassium ion channel thereby blocking flowout of potassium ion and inducing depolarization of the cell membrane, so that the calcium ion channels are opened to allow the extracellular calcium ions flow inwardly. The increase of intracellular calcium ions concentration triggers the release of the insulin._Sulphonylureas hypoglycemic drugs can be divided into two generations according to their time of coming into existence. The first generation includes tolpropamide, and the second generation includes glibenclamide (euglucan), gliclazide (diamicron), glipizide and gliquidone etc.

[0008] Biguanide hypoglycemic drugs inhibit appetite, improve the binding of insulin to the receptors, promote the anaerobic glycolysis in cells, inhibit tissue respiration and inhibit hepatic gluconeogenesis. The biguanide hypoglycemic drugs mainly include metformin, phenformin and buformin.

[0009] Other hypoglycemic drugs mainly include thiazolidinedione drugs (such as troglitazone, rosiglitazone, and pioglitazone, etc), ~~β3-adrenoceptor~~ β3-adrenoceptor regulators, glucagon receptor antagonists, fatty acid metabolism interfering agents, α-glycosidase inhibitors (such as acarbose, voglibose, miglitol), and aldose reductase inhibitors, etc.

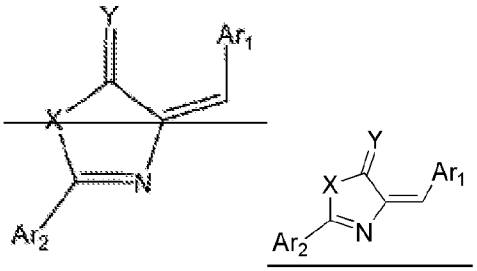
[0010] Recently, the development of research on glycometabolism related endogenous peptide hormone provides a new idea for the treatment of diabetes mellitus. When human body intakes nutrient materials, the enteroendocrine cells release enteropeptide hormone which mainly includes glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) and ~~regulates~~ regulate metabolism by affecting the insulin generation, gastrointestinal peristalsis, and islet cell proliferation. Wherein, GLP-1 is secreted by entero-pancreatic cells, and activates the adenylate cyclase to synthesize cA__MP by highly specifically binding to the GLP-1 receptor of β-islet cells, so as to further activate the protein kinase. The metabolic signal (glycometabolism) and kinase signal (binding of GLP-1) cooperate

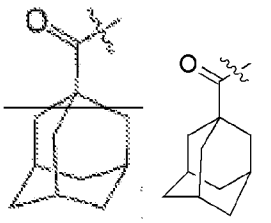
on the cell membrane level, which ~~to~~ finally cause the Ca^{2+} channel to open. The inward flowing of ~~and~~ the calcium ions ~~to flow inwardly so that~~ further stimulates the secretion of insulin ~~while inhibiting~~ and inhibits the generation of glucagon, thereby ~~decrease~~ decreases the postprandial blood glucose ~~to maintained~~ and maintain blood glucose concentration at a constant level. Also, GLP-1 has the function of neuroregulation, and can retard gastric emptying, and inhibit appetite. All of these are greatly beneficial for the control of diabetes mellitus. Normally, GLP-1 stimulates insulin secretion depending on the blood glucose concentration. As the blood glucose concentration decreases, the effect of GLP-1 on stimulating insulin secretion decreases. Therefore, the action of GLP-1 on decreasing blood glucose is self-limited, and ~~can not~~cannot cause ~~hypoglycaemia~~hypoglycemia. So, for treating diabetes mellitus, the medicaments with the GLP-1-like action are greatly desirable for the treatment of diabetes mellitus. GLP-1R agonists have been one researching focus of the international drug development organizations. At present, the researches on ~~GPL-1R~~ GLP-1R mainly focus on the polypeptide regulators. For example, AC 2993 of Amylin Corporation has been applied for clinic test in US (IND). AC2993 is a 39-amino acids polypeptide and has the effect of promoting the secretion of insulin as ~~GPL-1~~ GLP-1. Since the polypeptide drugs ~~is~~are inconvenient for oral administration and ~~is~~are readily to degrade, ~~non-peptides~~ non-peptide GLP-1R ~~regulator is~~ regulators are the new researching direction at present.

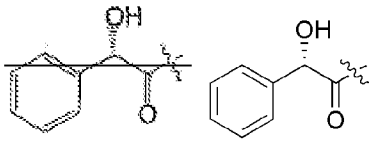
SUMMARY ~~DISCLOSURE~~ OF THE INVENTION

[0011] The object of the present invention is to design a group of novel small molecular organic ~~compound~~ compounds of substituted five-membered heterocyclic ring, ~~derivative~~ which may be used as glucagon-like peptide-1 receptor (GLP-1R) agonists, so as to prove a way for searching the ~~leader~~ lead compounds or the drugs for the medicaments against the diabetes mellitus. Another object of the present invention is to provide a process for preparing these compounds.

[0012] The Glucagon-like peptide-1 receptor agonists according the present invention have the specific structural formula as follows:



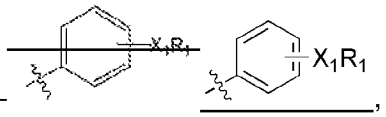
[0013] wherein, each of Ar_1 and Ar_2 independently is phenyl or substituted phenyl, and the substituent groups of the said substituted phenyl is one, two or three groups optionally selected from the following groups: alkyl; hydroxyl; substituted alkoxyl or alkylamino which contains subtitutent groups including halogen, alkoxyl, or hydroxyl; substituted alkanoylxy or alkanoylamino which contains the subtitutent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 alkenyl substituted with oxygen or amine, phenyl, benzyl, C_2-C_6 enoyl, C_3-C_6 cycloalkanoyl, benzoyl, substituted benzoyl which contains optional one, two, or three substituent groups including alkoxyl and alkylamino, benzyloyl, thenoyl, tert-butoxycarbonyl, adamantane formoxyl (

), and mandeloyl (

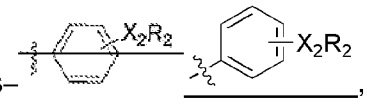
); alkoxyl; alkanoylamino; cycloalkoxyl; cycloalkanoylamino; amino; amide; alkoxycarbonyl; cycloalkoxycarbonyl; alkanoylxy; alkanoylamino; cycloalkanoylxy; cycloalkanoylamino; carbamido; urylene; alkanoyl; nitro ; carboxyl; and aldehyde group;

X is O, S, or NH; and

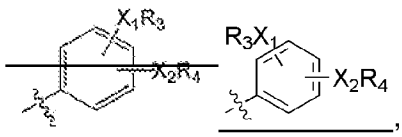
Y is O or S.

When Ar_1 is–  ,

[0014] wherein R_1 is any one of the following substituent groups: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl; benzoyl; tert-butoxycarbonyl; substituted benzoyl which contains optional one, two, or three substituent groups including alkoxyl and alkylamino; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; and when X_1 is O or NH,

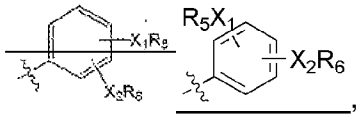
Ar_2 is–  ,

[0015] wherein R_2 is any one of the following substituent groups: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl; benzoyl; tert-butoxycarbonyl; substituted benzoyl which contains optional one, two or three substituent groups including alkoxyl and alkylamino; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; and X_2 is O or NH;

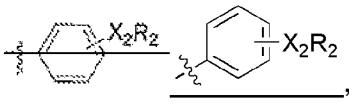
or Ar_2 is  ,

[0016] wherein each of R_3 and R_4 independently is any one of the following substituent groups: H; alkyl; substituted alkyl which contains the substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl; benzoyl; tert-butoxycarbonyl;

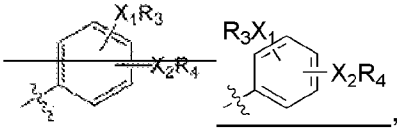
substituted benzoyl which contains optional one, two or three substituent groups including alkoxyl and alkylamino; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; and X_1 is O or NH; X_2 is O or NH.

When Ar_1 is  ,

[0017] wherein each of R_5 and R_6 independently is any one of the following substituent groups: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl; benzoyl; substituted benzoyl which contains optional one, two or three substituent groups including alkoxyl and alkylamino; tert-butoxycarbonyl; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; when X_1 is O or NH; and X_2 is O or NH,

Ar_2 is  ,

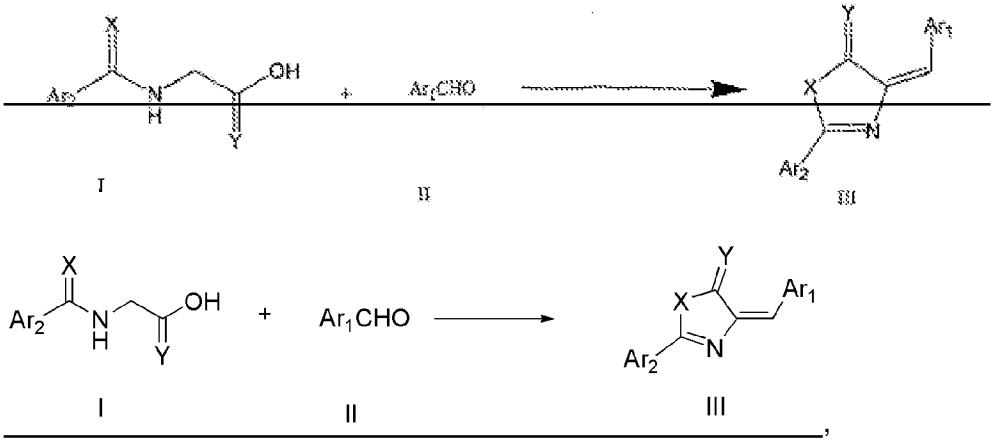
[0018] wherein R_2 is any one of the following substituent groups: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl, benzoyl, substituted benzoyl which contains optional one, two, or three substituent groups including alkoxyl and alkylamino; tert-butoxycarbonyl; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; and X_2 is O or NH;

or Ar_2 is  ,

[0019] wherein each of R_3 and R_4 independently is any one of the following substituent groups respectively: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl, benzoyl, substituted benzoyl which contains optional one, two, or three substituent groups including alkoxyl and alkylamino; tert-butoxycarbonyl; benzyloyl; thenoyl; adamantane formoxyl; and mandeloyl; and X_1 is O or NH; X_2 is O or NH.

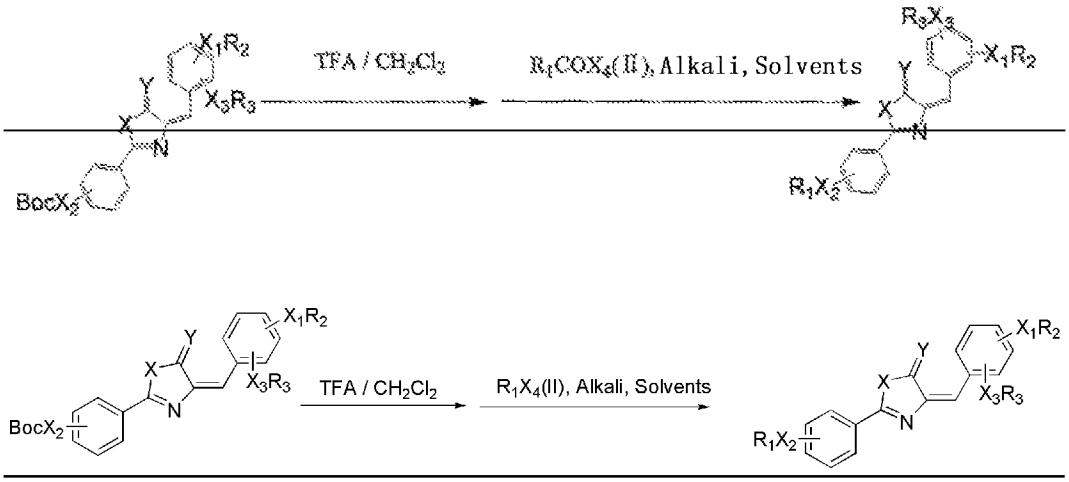
[0020] The present invention is performed by the following steps.

[0021] According to the chemical equation:



[0022] wherein each of Ar_1 and Ar_2 independently is phenyl or substituted phenyl, and the substituent groups of the said substituted phenyl is one, two or three groups optionally selected from the following group: nitro; carboxyl; aldehyde; tert-butoxycarbonyl and thenoyl substituted with oxygen or amino; X is O, S or NH; and Y is O or S.

[0023] Or, according to the following chemical equation:



[0024] wherein R_1, R_2 and R_3 are optional any one of the following substitutent group: H; alkyl; substituted alkyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 alkenyl; C_3-C_6 cycloalkyl; phenyl; benzyl; alkanoyl; substituted alkanoyl which contains substituent groups including halogen, alkoxyl, or hydroxyl; C_2-C_6 enoyl; C_3-C_6 cycloalkanoyl; benzoyl; tert-butoxycarbonyl; substituted benzoyl which contains ~~arbitrary~~ optional one, two, or three substituent groups including alkoxyl and alkylamino; benzyloyl; thenoyl; adamantane formoxyl; X is O, S, or NH; Y is O or S; each of X_1, X_2 and X_3 independently is O or NH; and X_4 is Cl or OH.

[0025] ~~The compound~~ Compound III is produced by the condensation reaction of ~~condensing the compounds~~ I ~~with~~ and II. And, the condensation is performed in the following solvent: dichloromethane, acetic anhydride, tetrahydrofuran, dimethylfuran, dichloroethane, toluene, benzene, water, dioxane or the mixture of the above solvents. If necessary, some activators may be added into the reaction, such as pyridine, N-methylmorpholine, isobutyl chloroformate, triethylamine, ~~diethylpropylethyl~~ diisopropylethyl amine, or DMAP etc. ~~According to reaction conditions of the compounds, the~~ The reaction temperature is generally ~~is~~ -78℃ to the room temperature (for example, for the compound ~~Wng462~~ Wang462 etc.), or is ~~50 —~~ 50° to

~~230—~~230° by heating (for example, for the compound ~~Wng520~~ Wang520 etc.). The reaction time is ~~determined~~ different according to the specific reactants. Generally, the reaction ~~progress~~ process is determined by tracing with TLC. After the completion of the reaction, the general post processing methods include filtrating with a pump, concentrating the reaction solution to remove the solvent, extracting and isolating with column chromatography etc. The final product III is verified with NMR detection.

[0026] The process for synthesizing the structural unit of the substituted five-membered heterocyclic ring of the present invention refers to Organic Syntheses, CV 2, 55.

[0027] The present invention designs and synthesizes the novel glucagon-like peptide-1 receptor (GLP-1R) agonists. The GLP-1R agonists of the present invention ~~have the good capability of binding~~ bind with the GLP-1R~~,~~ and promote the synthesis of cAMP, which may be used to prepare the medicaments for treating the glycometabolism disturbance~~-~~ related diseases such as type II diabetes, insensitivity to insulin and obesity etc. And, the defect of inconvenience for oral administration and being readily to degrade in the prior art, which exists in the medicaments with polypeptide regulators, can be overcame. ~~they can overcome the defect that the polypeptides regulator medicaments is inconvenient for oral amnistration and readily to degrade in the prior art. The~~Furthermore, the ~~—~~compounds of the present invention have ~~the~~ relative simple ~~structure~~ structures and are ~~readily~~ easy to ~~be prepared~~prepare.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] ~~The figure~~ Figure 1 shows the ~~detection result~~ detecting results of the expression of the report gene for the compounds of the present invention, which is used to evaluate the activating activity of the said compounds on GLP-1R. In ~~the~~

~~figure~~ Figure 1, the relative activity of the luciferase induced by 30nM of positive standard GLP-1 is regarded as 100%.

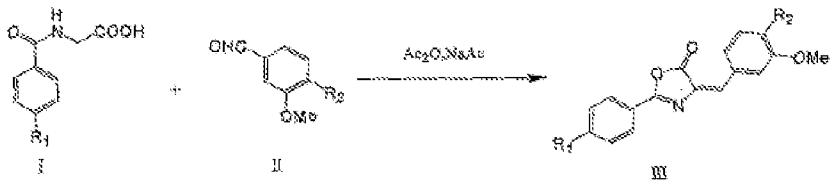
[0029] ~~The figure~~ Figure 2 shows the affection of the compound 2f on the concentration of cAMP in 293/GLP-1R cells.

DETAILED DESCRIPTION ~~EMBODIMENTS OF THE PRESENT INVENTION~~

[0030] The present invention will be further explained with reference to the following specific examples, but they don't limit the present invention in any way.

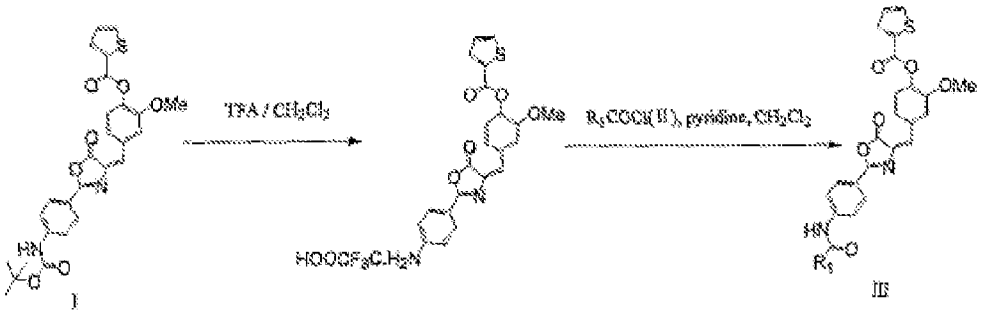
[0031] The preparation process for preparing the compounds in the following preparation examples 1 to 3 mainly includes three reaction operation procedures as ~~follow~~follows.

[0032] Procedure 1:



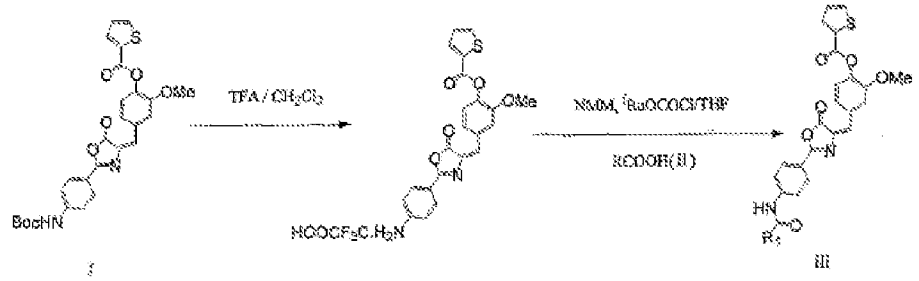
[0033] ~~The compounds~~ Compound I, ~~and~~ compound II, sodium acetate~~,~~ and acetic anhydride are mixed~~,~~ and heated to ~~melt~~ a molten state (ca. 150° to 230°~~150 to 230°C~~), and maintained ~~in the molten state~~ for 1 hour. Subsequently, ethanol is added into the reaction mixture and the resulted solution is cooled. The product is separated out ~~by~~ after crystallization ~~following by~~ and filtration. The ~~residue~~ residual liquid is concentrated to remove the solvents completely, and the product is isolated with column chromatography.

[0034] Procedure 2:



[0035] The compound I is dissolved in dichloromethane, and cooled in the cryohydrate bath at -20℃, ~~following~~ followed by adding trifluoroacetic acid. ~~and raising the temperature to~~ The solution is stirred at ~~the~~ room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the substrate is dissolved in dichloromethane, and cooled in the cryohydrate bath at ~~-20~~ -20°. Then, pyridine and acyl chloride are added orderly~~,~~. ~~the temperature is raised to the~~ The mixture is stirred at room temperature~~,~~ and ~~the reaction is~~ traced with TLC. ~~The reaction solution is concentrated, and~~ After concentration, the product is isolated with column chromatography.

[0036] Procedure 3:



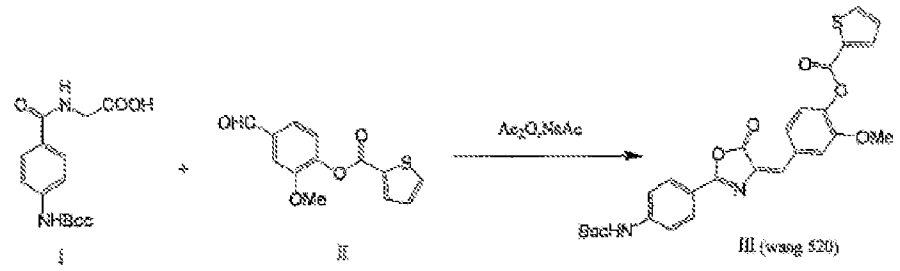
[0037] The compound I is dissolved in dichloromethane, and cooled in the cryohydrate bath at -20℃, followed ~~following~~ by adding trifluoroacetic acid. ~~and raising the temperature to~~ The solution is stirred at ~~the~~ room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely~~., following~~

~~by concentrating the reaction system~~The solution is concentrated and ~~removing~~ trifluoroacetic acid is removed completely. Then, the compound II is dissolved in tetrahydrofuran (THF), and cooled in the cryohydrate bath at ~~-20~~ -20°. Then, N-methylmorpholine (NMM) and $ClCOO^{i}Bu$ are added orderly. The reaction product of the compound I with trifluoroacetic acid is dissolved in tetrahydrofuran and then transferred into the above mixture with the syringe so as to react at ~~the~~ room temperature. The reaction is traced with TLC. After the reaction is completed, the reaction solution is concentrated, and the product is isolated with column chromatography.

[0038] For the products, the compounds wang520, wang337, wang405, wang450, wang520-1 and wang462-1 are prepared by the reaction procedure 1, the compounds wang420, wang462, wang524, wang516, wang488, ~~wang-568~~wang568, wang502, wang530, wang504, wang866, 2f, wang582, wang538, and wang496 are prepared by the procedure 2 with the compound wang520, and the compounds wang516-1~~,~~ and wang591 are prepared by the procedure 3 with the compound wang520.

[0039] In the following preparation examples, NMR is measured with Mercury-Vx 300M manufactured by Varian cooperation. NMR criteria are δH/C 7.26/7.77 ppm($CDCl_3$); δH/C 2.50/39.51ppm (DSMO-d6); and δH/C 3.31/49.15 ppm (Methyl-d3 Alcohol-d). The agents are provided by Shanghai Chemistry Agents Cooperation. And, the products are purified mainly by the column chromatography. The silica gel for separation is 200-300 mesh, and the model of the silica gel for the column chromatography is thick and hollow (ZLX-II), and is produced by the branch factory of Qingdao Haiyang Chemical plant.

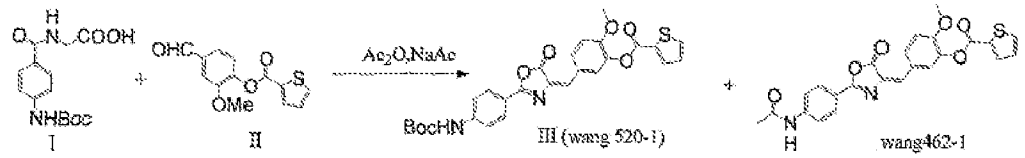
[0040] Example 1



I II III (wang 520)

[0041] At ~~the~~ room temperature, the compound II (466mg, 1.78mmol), the compound I (576 mg, 1.96 mmol), sodium acetate (146mg, 1.78 mmol) and 2mL of acetic anhydride are mixed~~, following by heating~~ and heated to 170℃. ~~until the system melts, and maintaining~~ The reaction is maintained in the molten state for 1 hour. Then, 2mL of ethanol is added into the resultant mixture, and the reaction is then cooled to ~~the~~ room temperature. ~~So the yellow~~ Yellow solids are separated out and filtered. The ~~residue~~ residual liquid is concentrated~~,~~ and the solvent is removed completely~~,~~ to obtain the crude product~~. The crude product is chromatographed~~, which is isolated over silica gel column with petroleum ether/ethyl acetate (5:1 v/v) to obtain 556 mg of ~~product,~~ the compound wang520 (yield: 60%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (s, 9H), 3.95 (s, 3H), 6.79 (br, 1H), 7.16 (s, 1H), 7.20 (dd, *J* = 4.8 Hz, 3.9 Hz, 1H), 7.25 (d, *J* = 9.9 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.63 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 7.69 (dd, *J* = 4.8 Hz, 1.2 Hz, 1H), 8.02 (dd, *J* = 3.9 Hz, 1.2 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 2H), 8.17 (d, *J* = 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.17, 55.79, 81.23, 115.28, 117.92, 119.11, 123.09, 125.74, 128.02, 129.29, 129.41, 132.18, 132.75, 133.29, 133.71, 134.99, 141.57, 143.46, 151.37, 152.08, 159.93, 163.13, 167.46。



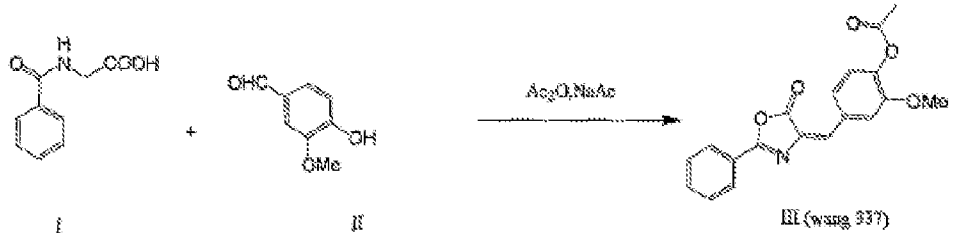
I II III (wang 520-1)

[0042] At ~~the~~ room temperature, the compound II (466mg, 1.78mmol), the compound I (576 mg, 1.96 mmol), sodium acetate (146mg, 1.78 mmol) and 2mL of

acetic anhydride are mixed~~, following by heating~~ and heated to 200℃. ~~until the system melts, and maintaining~~ The reaction is maintained in the molten state for 1 hour. Then, 2mL of ethanol is added into the resultant mixture, and then the reaction is cooled to ~~the~~ room temperature. The ~~liquid~~ solution is concentrated~~, and the solvent is removed completely,~~ to obtain the crude product~~. The crude product is chromatographed~~, which is isolated over silica gel column with petroleum ether/ethyl acetate (1:1 v/v) to obtain 158 mg of the compound wang462-1.

^{1}H NMR (300 MHz, $CDCl_3$, wang520-1) δ 1.50 (s, 9H), 3.88 (s, 3H), 7.27 (s, 1H), 7.33-7.37 (2H), 7.69 (d, J = 8.7 Hz, 2H), 8.01 (d, J = 8.7 Hz, 2H), 8.07 (d, J = 3.9 Hz, 1H), 8.13 (d, J = 4.8 Hz, 1H), 8.22-8.26 (2H), 9.93 (s, 1H).

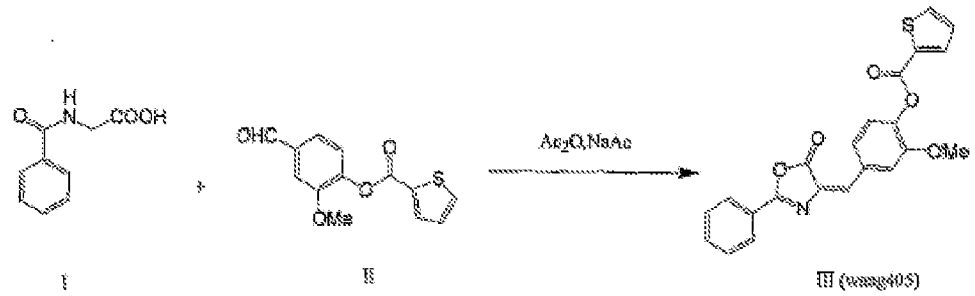
^{1}H NMR (300 MHz, $CDCl_3$, wang462-1) δ 2.22 (s, 3H), 3.91 (s, 3H), 7.07 (d, J = 8.7 Hz, 1H), 7.14 (s, 1H), 7.21 (m, 1H), 7.42 (m, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 4.8 Hz, 1H), 7.99 (d, J = 8.7 Hz, 1H), 8.05 (m, 1H), 8.10 (d, J = 8.4 Hz, 2H), 8.19 (m, 1H).



I II III (wang 337)

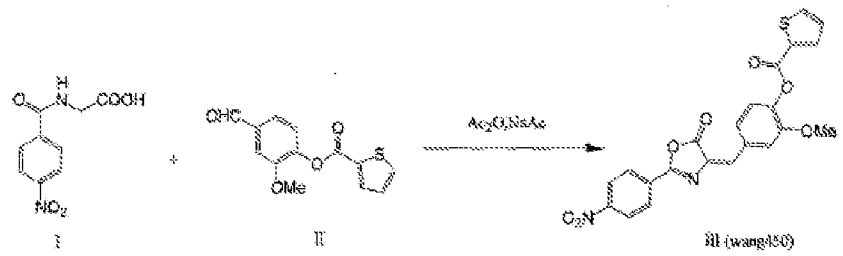
[0043] At ~~the~~ room temperature, the compound II (1.46g, 9.6mmol), the compound I (1.9 g, 10.7 mmol), sodium acetate (0.8 g, 9.8 mmol) and 2.8mL of acetic anhydride are mixed~~, following by heating~~ and heated to 170℃. ~~until the system melts, and maintaining~~ The reaction is maintained in the molten state for 1 hour. Then, 5mL of ethanol is added into the resultant mixture, and then the reaction is cooled to ~~the~~ room temperature. ~~So the~~ The yellow solids are separated out and filtered to obtain 2.0 g ~~of product, the~~ the compound wang337 (yield: 62%).

^{1}HNMR (300MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.97 (s, 3H), 7.13 (d, J = 8.4 Hz, 1H), 7.20 (s, 1H), 7.50-7.56 (2H), 7.59-7.65 (2H), 8.12-8.15 (3H).



[0044] At ~~the~~ room temperature, the compound II (262mg, 1.0mmol), the compound I (200 mg, 1.1 mmol), sodium acetate (82mg, 1.0 mmol) and 1mL of acetic anhydride are mixed~~, following by heating~~ and heated to 170℃. ~~until the system melts, and maintaining~~ The reaction is maintained in the molten state for 1 hour. Then, 5mL of ethanol is added into the resultant mixture, and then the reaction is cooled to ~~the~~ room temperature. ~~So the~~ The yellow solids are separated out and filtered. The ~~residue~~ residual liquid is concentrated~~,~~ and the solvent is removed completely~~,~~ to obtain the crude product~~. The crude product is chromatographed~~, which is isolated over silica gel column with petroleum ether/ethyl acetate (6:1 v/v) to obtain 235 mg of ~~product,~~ the compound wang405 (yield: 58%).

^{1}HNMR (300MHz, $CDCl_3$) δ 3.97 (s, 3H), 7.20 (dd, J = 4.8 Hz, 3.9 Hz, 1H), 7.24 (s, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.51-7.57 (2H), 7.60-7.70 (3H), 8.02 (dd, J = 3.6 Hz, 0.9 Hz, 1H), 8.14-8.19 (3H).

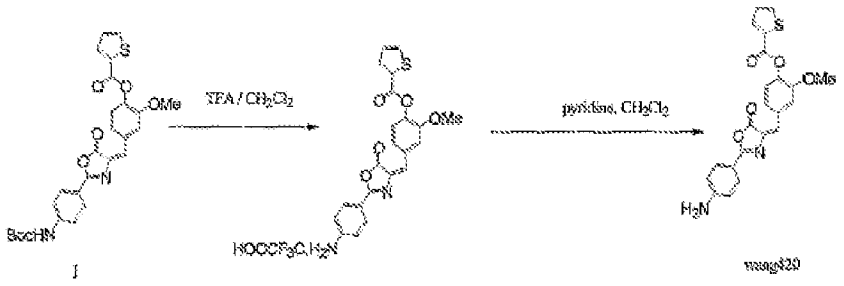


[0045] At ~~the~~ room temperature, the compound II (262mg, 1.0 mmol), the compound I (250 mg, 1.1 mmol), sodium acetate (82mg, 1.0 mmol) and 4mL of acetic anhydride are mixed~~, following by heating, and maintaining the system at~~ and heated to 210 to 230℃. The reaction is maintained in the molten state for 1 hour. Then, 5mL of

ethanol is added into the resultant mixture, and then the reaction is cooled to ~~the~~ room temperature. ~~So the~~ The yellow solids are separated out and filtered to obtain 100mg of ~~product,~~ the compound wang450 (yield: 22%).

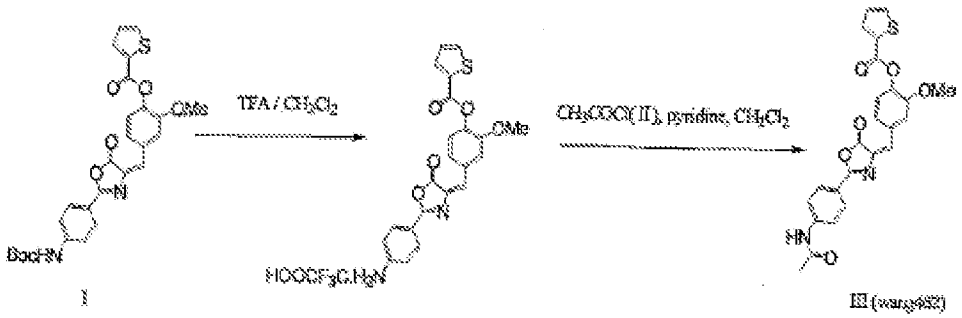
^{1}HNMR (300MHz, $CDCl_3$) δ 3.97 (s, 3H), 7.21 (dd, J = 4.8 Hz, 3.9 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.37 (s, 1H), 7.70 (d, J = 5.1 Hz, 1H), 7.73 (dd, J = 9.9 Hz, 1.5 Hz, 1H), 8.02 (d, J = 3.9 Hz, 1H), 8.09 (d, J = 1.8 Hz, 1H), 8.33 (d, J = 9.0 Hz, 2H), 8.40 (d, J= 9.3Hz, 2H)。

[0046] Example 2



[0047] The compound I (50mg, 0.1mmol) is dissolved ~~into~~in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at -20℃, followed ~~following~~ by ~~adding~~ 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to~~ The reaction is stirred at ~~the~~ room temperature ~~. And, the reaction is~~and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, then the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20~~ -20°, ~~following~~ followed by adding 40 μL (0.6mmol) of pyridine~~, and gradually raising the temperature to~~ The reaction is stirred at ~~the~~ room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (2:1 v/v) to obtain 38 mg of ~~product,~~ the compound wang420 (yield: 90%).

^{1}HNMR (300MHz, $CDCl_3$) δ 3.94 (s, 3H), 7.20-7.24 (m, 2H), 7.27 (d, J = 1.8 Hz, 1H), 7.66 (dd, J = 8.1 Hz, 1.5 Hz, 1H), 7.71 (dd, J = 4.8 Hz, 0.9 Hz, 1H), 7.76 (d, J = 9.0 Hz, 2H), 8.03 (dd, J = 3.6 Hz, 0.9 Hz, 1H), 8.07 (d, J = 1.5 Hz, 1H), 8.14 (d, J = 8.7 Hz, 2H), 8.20 (br, 2H).

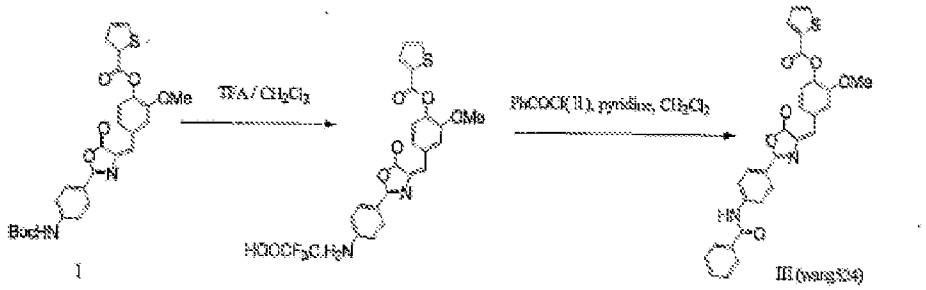


I

III (wang462)

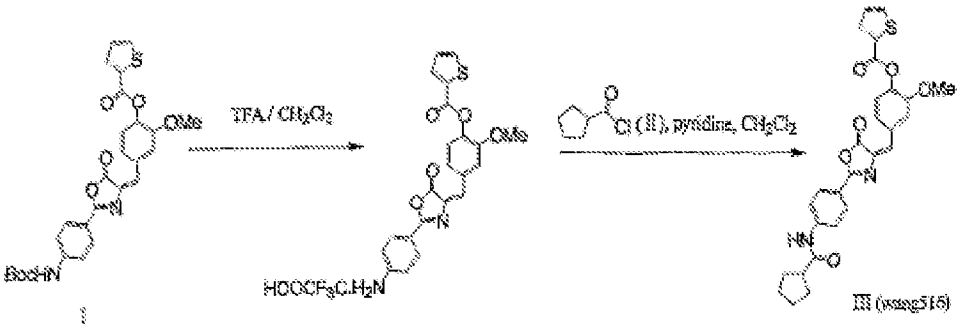
[0048] The compound I (50mg, 0.1mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at -20℃, followed ~~following~~ by ~~adding~~ 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20~~ -20°, ~~following~~ followed by adding 40 μL (0.6mmol) of pyridine~~, adding the~~ and compound II (27μL, 0.39mmol). ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (1.5:1 v/v) to obtain 26mg of ~~product,~~ the compound wang462 (yield: 56%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (s, 3H), 3.88 (s, 3H), 7.12 (s, 1H), 7.20-7.24 (m, 2H), 7.55 (d, J = 1.5 Hz, 1H), 7.60 (d, J = 9.0 Hz, 2H), 7.71 (dd, J = 4.8 Hz, 0.9 Hz, 1H), 7.77 (br, 1H), 7.97 (d, J = 8.7 Hz, 2H), 8.03 (dd, J = 3.9 Hz, 0.9 Hz, 1H), 8.07 (d, J = 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.66, 55.84, 155.64, 119.55, 120.54, 123.35, 126.15, 128.43, 129.59, 129.87, 132.37, 133.12, 133.52, 134.26, 135.41, 141.85, 143.13, 151.63, 160.63, 163.28, 167.60,168.99.



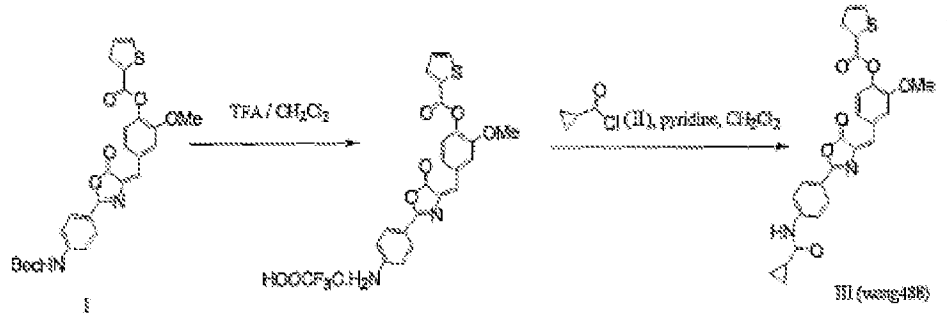
[0049] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at -20℃ ~~following~~ followed by ~~adding~~ 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~ The solution is stirred at room temperature~~. And, the reaction is~~and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20~~ -20°, ~~following~~ followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (23μL, 0.2mmol). ~~and gradually raising the temperature to the~~ The mixture is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (5:1 v/v) to obtain 15mg of ~~product,~~ the compound wang524 (yield: 36%).

^{1}HNMR (300MHz, DMSO-d_6) δ 3.90 (s, 3H), 7.22 (d, J = 5.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.39-7.44 (1H), 7.50-7.58 (2H), 7.83 (d, J = 8.4 Hz), 7.98 (d, J = 8.7 Hz, 2H), 8.04-8.22 (7H), 10.74 (s, 1H).



[0050] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at -20℃ ~~following~~ followed by ~~adding~~ 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 , following~~ -20° followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (25μL, 0.2mmol). ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (4:1 v/v) to obtain 25mg of ~~product,~~ the compound wang516 (yield: 62.5%).

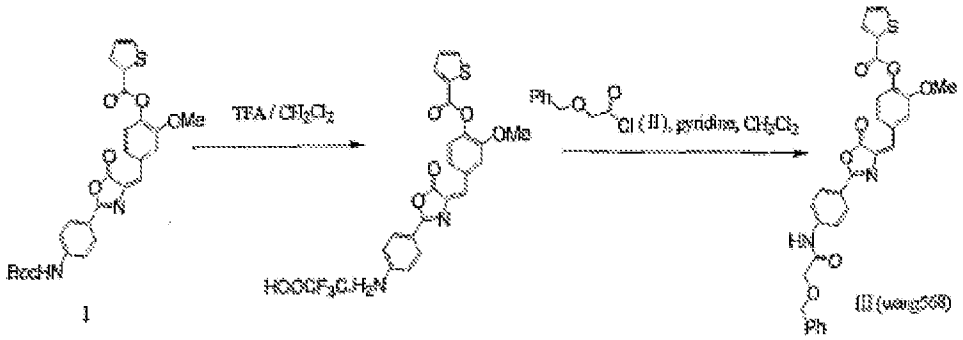
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57 (m, 2H), 1.63-1.77 (m, 4H), 1.80-1.89 (m, 2H), 2.84 (m, 1H), 3.89 (s, 3H), 7.31 (m, 2H), 7.40 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 9.0 Hz, 2H), 7.94 (dd, J = 8.4 Hz, 1.8 Hz, 1H), 8.03 (dd, J = 3.9 Hz, 1.2 Hz, 1H), 8.07 (d, J = 9.0 Hz, 2H), 8.10 (dd, J = 4.8 Hz, 1.2 Hz, 1H), 8.18 (d, J = 1.8 Hz, 1H), 10.35 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.62, 30.00, 55.97, 115.74, 118.71, 119.04, 123.52, 125.27, 128,51, 128.77, 129.24, 131.19, 132.78. 133.34, 135.43, 135.50, 140.86, 144.42, 151.04, 159.24, 162.91, 166.93, 175.11。



[0051] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at -20℃ ~~following by adding~~ followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~

The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 , following~~ -20°, followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (23μL, 0.2mmol)~~. and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (4:1 v/v) to obtain 25mg of ~~product,~~ the compound wang488 (yield: 64%).

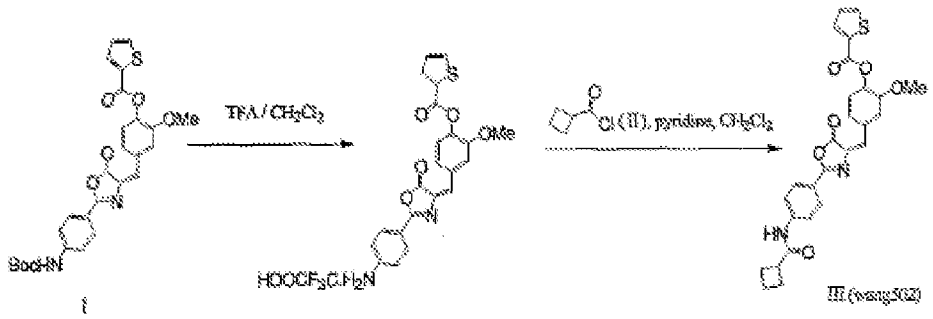
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (m, 2H), 0.85 (m, 2H), 1.84 (m, 1H), 3.88 (s, 3H), 7.28 (s, 1H), 7.32 (dd, J = 5.1 Hz, 3.9 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.92 (dd, J = 8.4 Hz, 1.5 Hz, 1H), 8.04 (m, 1H), 8.05 (d, J = 8.7 Hz, 2H), 8.11 (dd, J = 4.8 Hz, 1.2 Hz, 1H), 8.18 (d, J = 1.8 Hz, 1H), 10.68 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.78, 14.83, 55.97, 115.71, 118.73, 118.93, 123.53, 125.32, 128.54, 128.81, 129.32, 131.22, 132.80, 133.36, 135.46, 135.53, 140.88, 144.24, 151.05, 159.29, 162.91, 166.96, 172.44。



[0052] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid~~. and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane~~,~~ and cooled in the cryohydrate bath at ~~-20 ,~~

~~following~~ ~~-20°, followed~~ by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (23μL, 0.2mmol)~~. The reaction is stirred at and gradually raising the temperature to the~~ room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (4:1 v/v) to obtain 26mg of ~~product,~~ the compound wang568 (yield: 57%).

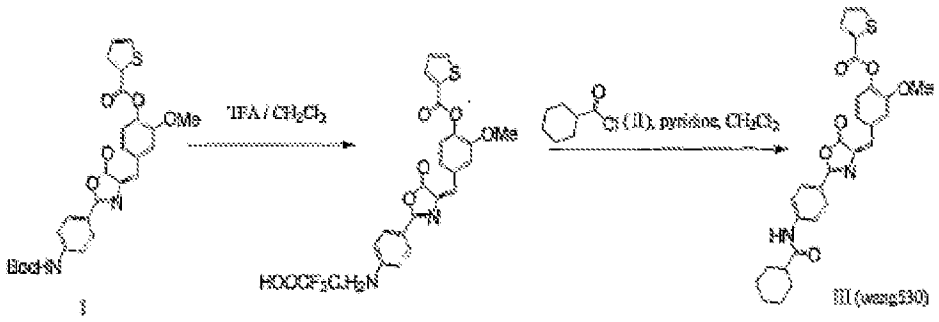
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (s, 3H), 4.13 (s, 2H), 4.68 (s, 2H), 7.18 (s, 1H), 7.19-7.26 (m, 2H), 7.39-7.50 (m, 5H), 7.63 (dd, J = 6.9 Hz, 0.9 Hz, 1H), 7.69 (dd, J = 4.8 Hz, 0.9 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 8.01 (dd, J = 3.6 Hz, 1.2 Hz, 1H), 8.10 (d, J = 8.7 Hz, 2H), 8.16 (d, J = 1.5 Hz, 1H), 8.56 (s, 1H).



[0053] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid~~, and gradually raising the temperature to the~~ The reaction is at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 , following~~ -20° followed by adding 40 μL (0.6mmol) of pyridine~~, adding the~~ and compound II (23μL, 0.2mmol)~~. and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (4:1 v/v) to obtain 22mg of

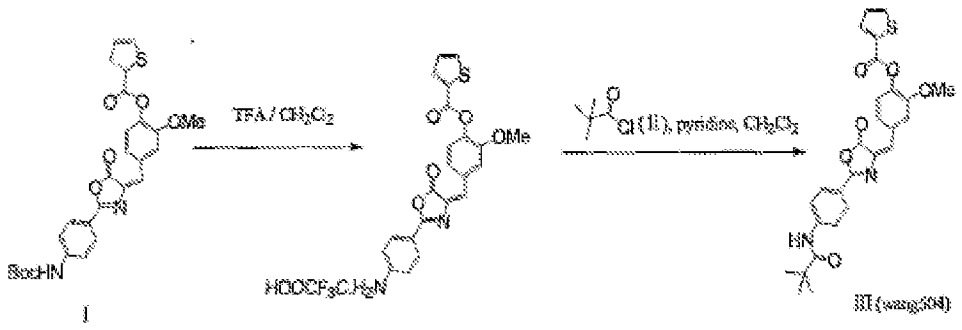
product, the compound wang502 (yield: 56%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.81-1.94 (m, 2H), 2.12-2.28 (m, 4H), 3.29 (m, 1H), 3.89 (s, 3H), 7.31 (s, 1H), 7.33 (m, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 8.1 Hz, 2H), 8.04-8.08 (2H), 8.12 (d, J = 5.1 Hz, 1H), 8.19 (s, 1H), 10.20 (s, 1H).



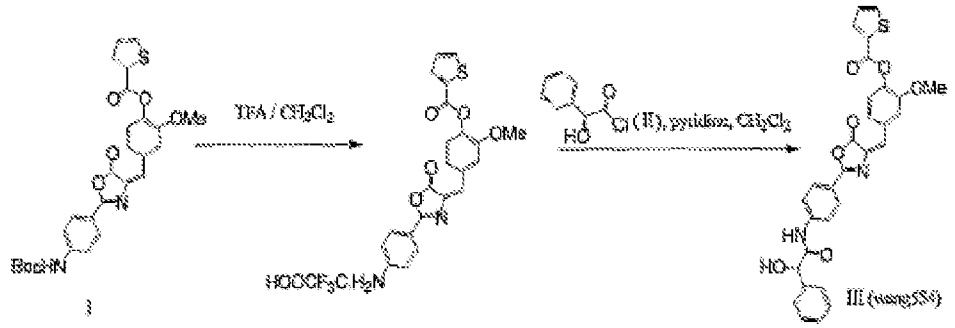
[0054] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 ,~~ ~~following~~ -20° followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (23μL, 0.2mmol). ~~and gradually raising the temperature to the~~ The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~ After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (4:1 v/v) to obtain 24mg of ~~product,~~ the compound wang530 (yield: 57%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20-1.48 (6H), 1.65-1.81 (4H), 2.39 (m, 1H), 3.89 (s, 3H), 7.32 (s, 1H), 7.34 (m, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.95 (d, J = 8.1 Hz, 1H), 8.04 (m, 1H), 8.08 (d, J = 8.7 Hz, 2H), 8.12 (d, J = 4.8 Hz, 1H), 8.20 (m, 1H), 10.31 (s, 1H).



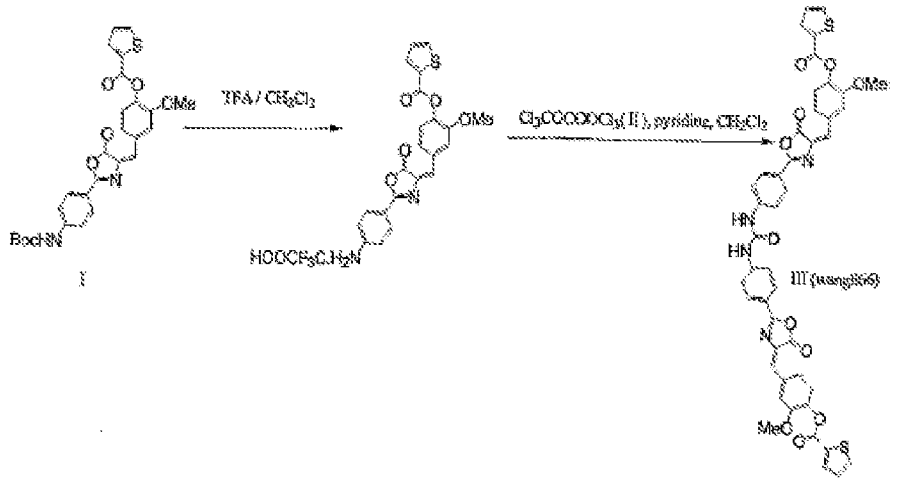
[0055] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 , following~~ -20° followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (23μL, 0.2mmol). ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (6:1 v/v) to obtain 4mg of ~~product,~~ the compound wang504 (yield: 10%).

^{1}H NMR (300 MHz,$CDCl_3$) δ 1.34 (s, 9 H), 3.94 (s, 3H), 7.15 (s, 1H), 7.20 (dd, J = 4.8 Hz, 3.6 Hz, 1H), 7.23 (s, 1H), 7.58 (br, 1H), 7.64-7.69 (2H), 7.72 (d, J = 8.7 Hz, 2H), 8.02 (dd, J = 3.6 Hz, 1.5 Hz, 1H), 8.08 (d, J = 9.0 Hz, 2H), 8.11 (d, J = 1.8 Hz, 1H).



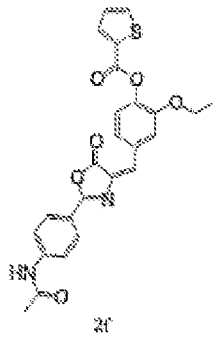
[0056] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane~~,~~ and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 ,~~ ~~following~~ -20°, followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~ and the compound II (27μL, 0.2mmol). ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. The reaction is~~ and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~After concentration the crude product is obtained, which is isolated ~~. The crude product is chromatographed~~ over silica gel column with CH_2Cl_2 to obtain 40mg of ~~product,~~ the compound wang554 (yield: 89%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 3H), 6.28 (s, 1H), 7.05 (s, 1H), 7.16 (d, J = 8.1 Hz, 1H), 7.20 (dd, J = 5.1 Hz, 3.6 Hz, 1H), 7.39-7.41 (2H), 7.50-7.55 (3H), 7.60 (d, J = 9.0 Hz, 2H), 7.71 (dd, J = 5.1 Hz, 1.2 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 1.2 Hz), 8.03 (dd, J = 3.6 Hz, 0.9 Hz, 2H), 8.24 (s, 1H), 8.42 (s, 1H).



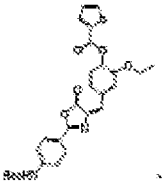
[0057] The compound I (52mg, 0.1mmol) is dissolved ~~into~~ in 2mL of dichloromethane~~,~~ and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. After concentrating the reaction system and removing trifluoroacetic acid completely, the reaction intermediate is dissolved in 2 mL of dichloromethane, and cooled in the cryohydrate bath at ~~-20 ,~~ ~~following~~ -20°, followed by adding 40 μL (0.6mmol) of pyridine~~, adding~~and the compound II (10mg, 0.03mmol). ~~and gradually raising the temperature to the~~The solution is stirred at room temperature~~. The reaction is~~and traced with TLC. ~~The reaction solution is concentrated, and the solvents are removed to obtain~~After concentration the crude product is obtained, which is isolated~~. The crude product is chromatographed~~ over silica gel column with CH_2Cl_2 to obtain 19mg of ~~product,~~ the compound wang866 (yield: 44%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (s, 6H), 7.33 (dd, J = 4.8 Hz, 3.9 Hz, 2H), 7.36 (s, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.93 –7.96 (2H), 7.96 (d, J = 8.7 Hz, 4H), 8.04 (dd, J = 3.3 Hz, 0.9 Hz, 2H), 8.12 (dd, J = 4.8 Hz, 0.9 Hz, 2H), 8.17 (d, J = 8.7 Hz, 4H), 8.20 (d, J = 1.8 Hz, 2H), 11.66 (s, 2H).



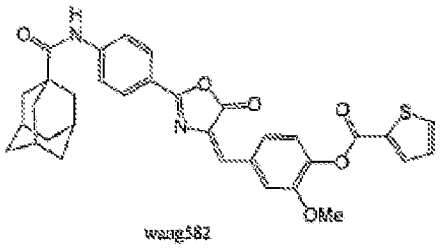
2f

[0058] ~~Accord~~ According to the same ~~process~~procedure, the compound 2f is prepared in 56% yield ~~by using the reaction product of~~from 1eq of compound



, ~~with trifluoroacetic acid~~ and 1.5eq of acetyl chloride ~~(yield: 56%)~~.

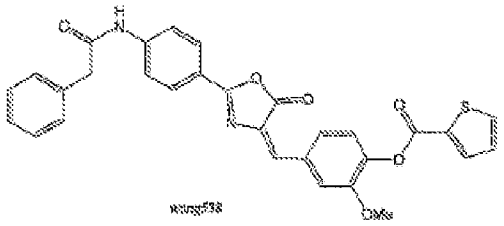
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (t, J = 6.9 Hz, 3H), 2.24 (s, 3H), 4.18 (q, J = 6.9 Hz, 2H), 7.11 (s, 1H), 7.19 (m, 1H), 7.45 (m, 2H), 7.62-7.70 (4H), 8.02 (m, 1H), 8.08 (d, J = 9.0 Hz, 2H).



wang582

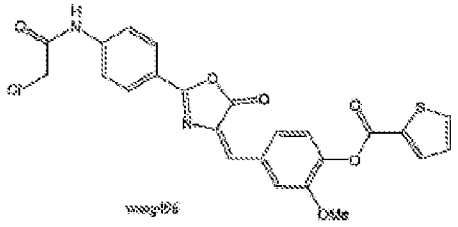
[0059] According to the same ~~process~~procedure, the compound wang582 is prepared ~~by using the reaction product of~~in 38% yield from 1eq of the compound wang520 ~~with trifluoroacetic acid~~ and 1.5eq of diamantane formyl chloride ~~(yield: 38%)~~.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (6H), 1.99 (6H), 2.12 (3H), 3.95 (s, 3H), 7.14-7.23 (2H), 7.54 (s, 1H), 7.61-7.70 (2H), 7.73 (d, J = 9.0 Hz, 2H), 8.02 (dd, J = 3.9 Hz, 1H), 8.09 (d, J = 9.0 Hz, 2H), 8.12 (d, J = 1.8 Hz, 1H).



[0060] According the same ~~process~~procedure, the compound wang538 is prepared ~~by using the reaction product of~~in 58% yield from 1eq of the compound wang520 ~~with trifluoroacetic acid~~ and 1.5eq of benzyl acetyl chloride ~~(yield: 58%)~~.

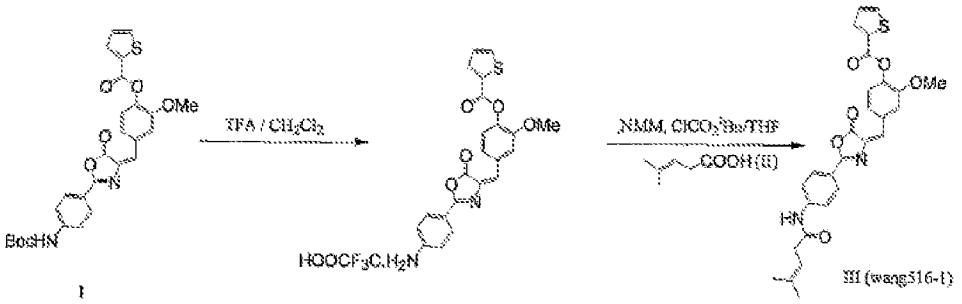
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 2H), 3.92 (s, 3H), 7.16 (s, 1H), 7.19-7.24 (2H), 7.34-7.74 (6H), 7.59 (d, J = 8.7 Hz, 2H), 7.62 (m, 1H), 7.70 (d, J = 4.8 Hz, 1H), 8.02 (d, J = 8.7 Hz, 2H), 8.13 (m, 1H).



[0061] According the same ~~process~~procedure, the compound wang496 is prepared ~~by using the reaction product of~~in 70% yield from 1eq of the compound wang520 ~~with trifluoroacetic acid~~ and 1.5eq of chloro acetyl chloride ~~(yield: 70%)~~.

^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (s, 3H), 4.36 (s, 2H), 7.34 (s, 1H),7.41 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 9.0 Hz, 2H), 7.93-7.98 (2H), 8.05 (m, 1H), 8.12 (d, J = 7.5 Hz, 2H), 8.22 (m, 1H), 8.89 (m, 1H), 10.95 (s, 1H).

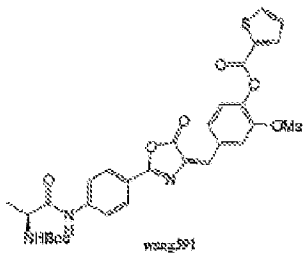
[0062] Example 3



[0063] The compound I (40mg, 0.08mmol) is dissolved ~~into~~ in 2mL of dichloromethane, and ~~is~~ cooled in the cryohydrate bath at ~~-20 following by adding~~ -20° followed by 1mL of trifluoroacetic acid. ~~and gradually raising the temperature to the~~The reaction is stirred at room temperature~~. And, the reaction is~~ and traced with TLC until the compound I is reacted completely. ~~After concentrating the reaction system and removing~~The solution is concentrated and trifluoroacetic acid is removed completely.~~,~~ In another dry 10mL round-bottomed flask, the compound II (19 μL, 0.16mmol) is dissolved in 2 mL of tetrahydrofuran, and cooled in the cryohydrate bath at ~~-20~~ -20° with stirring for 10min at this temperature. Then, N-methylmorpholine (NMM) (53 μL, 0.48mmol) and ClCOO^{i}Bu (21 μL, 0.16mmol) are added orderly with stirring for 0.5 hour at ~~-20~~ -20°. The ~~reaction product~~ residue of the compound I with trifluoroacetic acid is dissolved in 1mL of tetrahydrofuran and then –transferred into the above mixture with the syringe so as to react at the room temperature for about 15 hours. ~~The reaction solution is concentrated and the solvents are removed completely to obtain~~After concentration the crude product is obtained, which is isolated~~. The crude product is chromatographed~~ over silica gel column with petroleum ether/ethyl acetate (5:1 v/v) to obtain

12mg of ~~product,~~ the compound wang516-1(yield: 30%).

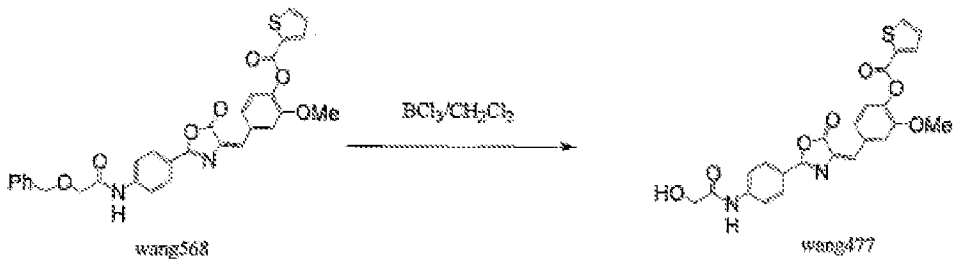
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74 (s, 3H), 1.87 (s, 3H), 3.18 (d, J = 7.8 Hz, 2H), 3.95 (s, 3, H), 5.42 (m, 1H), 7.19 (s, 1H), 7.20-7.27 (2H), 7.63 (2H), 7.65 (d, J = 1.8 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 8.02 (dd, J = 3.6 Hz, 0.9 Hz, 1H), 8.09 (d, J = 9.0 Hz, 2H), 8.16 (d, J = 2.1 Hz, 1H).



[0064] According to the same ~~process~~procedure, the compound wang591 is

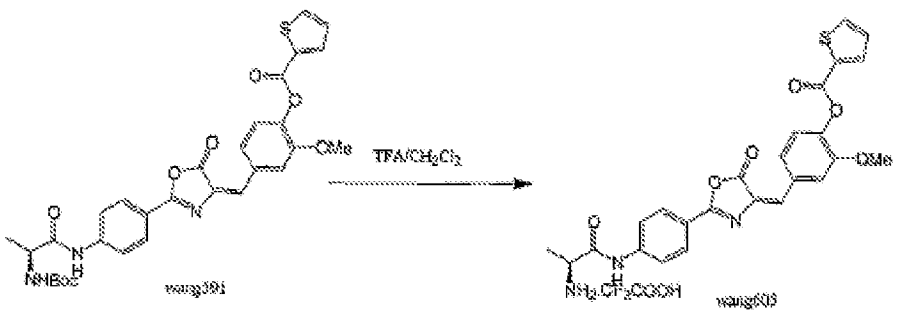
prepared ~~by using the reaction product of~~in 18% yield from 1eq of the compound wang520 ~~with trifluoroacetic acid~~ and 2.0eq of the compound Boc-Ala-OH ~~(yield: 18%)~~.

[0065] Example ~~14~~4



[0066] The compound wang568 (11mg, 0.02mmol) is dissolved ~~into~~ in 2mL of dichloromethane~~,~~ and cooled at ~~-78~~ -78° for 10 minutes, ~~following by adding~~ followed by 0.2mL 1M of BCl_3 ~~/~~in n-hexane solution ~~(1M)~~ to continue ~~reacting~~ stirring for 30 minutes at ~~-78~~ -78°. Then, the temperature is raised to ~~-18~~ -18° to react for 4 hours. 2mL of ether is added to quench the reaction with stirring for 30 minutes at the room temperature, ~~following~~ followed by adding 5mL of water. The water phase and the organic phase are separated. The water phase is extracted with dichloromethane~~, and the~~. The organic phase is combined~~,~~ and dried with anhydrous $MgSO_4$~~, and concentrated. The~~After concentration the crude product is obtained, which is isolated over column chromatography with petroleum ether/ethyl acetate (1/2, v/v) to obtain the compound wang477 (1.5mg, yield: 17%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (br, 1H), 3.95 (s, 3H), 4.26 (s, 2H), 7.18 (s, 1H), 7.20 (dd, *J* = 8.7 Hz, 4.8 Hz, 1H), 7.23 (d, *J* = 3.3 Hz, 1H), 7.63 (d, *J* = 8.1 Hz, 1H), 7.71 (dd, *J* = 5.1 Hz, 1.5 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 2H), 8.02 (d, *J* = 3.6 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 2H), 8.14 (m, 1H), 8.57 (b r, 1H).



[0067] The compound wang591 (3mg) is dissolved ~~into~~ in 1.5 mL of dichloromethane, and cooled in ice bath for 5 minutes~~, following by adding~~ followed by 0.15 mL of trifluoroacetic acid. Then, the temperature is gradually raised to the room temperature, and the reaction is traced with TLC. After the raw material disappears, the solvent and trifluoroacetic acid are removed ~~by pumping~~in vacuum to obtain 2mg of ~~the product,~~ the compound wang-605 (yield: 65%).

^{1}H NMR (300 MHz, Methyl-d_3 Alcohol-d) δ 1.63 (d, J = 7.2 Hz, 3H), 3.95 (s, 3H), 4.09 (m, 1H), 7.265 (s,1H), 7.267 (d, J = 8.7 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H),7.81 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.87 (d, J = 9.0 Hz, 2H), 7.91 (dd, J = 5.1 Hz, 1.2 Hz, 1H), 8.01 (dd, J = 3.6 Hz, 0.9 Hz, 1H), 8.16 (d, J = 9.3 Hz, 2H), 8.25 (d, J = 2.1 Hz, 1H).

[0068] Example ~~4 Experiments testing~~ 5 Experimental test on biological activity

[0069] 1. Testing the expression of the report gene

[0070] Upon that GLP-1R binds to GLP-1 or agonists, its Gα_subunit is activated to stimulate the adenylate cyclase, which makes the increase in the concentration of intracellular cAMP. Since the promoter region of the proinsulin gene has the cAMP response element, upon binding of cAMP to this response element, the transcription of the proinsulin gene is activated, so as to increase the sensitivity of β- islet cells to glucose~~,~~ and improve the expression and secretion of insulin (Diabetes, 2000, vol.49: 1156-1164). The screening model employs the human embryonal nephric cell strain which is stably transfected with the expression vector of GLP-1R gene and the expression vector of luciferase report gene under the regulation of cAMP response element, to detect its response to the candidate compound (Cell Biology, 1992, Vol.89:

8641-8645; Proc. Natl. Acad. Sci. U.S.A. 1987, Vol.84: 3434-3438). When screening the candidate compounds, the compounds which may induce the luciferase report gene to express have the activity of activating GLP-1.

[0071] 1.1 Experimental material and instruments

[0072] Cell strain: HEK 293/GLP1R+Luc strain which stably express GLP-1R and luciferase (National New Medicaments Screening Center)

[0073] Fetal calf serum (GIBCO/BRL Cooperation)

[0074] Steady-glo™ luciferase analysis system (Promega Cooperation)

[0075] Standard GLP-1 (Sigma Cooperation)

[0076] G418 (Invitrogen Cooperation)

[0077] Forma carbon dioxide incubator (Forma Cooperation)

[0078] Victor 2 counting machine (Wallac Cooperation)

[0079] Candidate compound: the compounds wang524, wang520, wang462, 2f, wang516, wang516-2, wang502 and wang504;

[0080] 1.2 Experimental process

[0081] HEK 293/GLP1R+Luc cell in 20000 cells/100μl/well are inoculated into 96-well plate, ~~are~~ cultured at ~~37~~ 37° overnight with DMEM culture medium containing 10% of fetal calf serum and 500μg/mL of G418. The candidate compounds wang516-2, wang502, and wang504 are respectively diluted to 2mM, 1mM, 0.3mM, 0.1mM, 0.03mM, 0.01mM, and 0.003mM, and the other candidate compounds are diluted gradually from 30mM for 8 times by a ratio of 1:3 to get a concentration gradient (i.e., 30mM, 10mM, 3mM, 1mM, 0.3mM, 0.1mM, 0.03mM, and 0.01mM), ~~following by being~~ which is added into the above 96-well plate at 1μl/well. Then, the cells are cultured at 37 in 5% of CO_2 for 6 hours. The activity of luciferase is

detected according to the specification of Steady-glo™ luciferase analysis system kit, and counting is performed with Victor 2 counting machine. The positive control adopts 30nM of standard GLP-1.

[0082] 1.3 Experimental result

[0083] The experimental result of the report gene for the candidate compounds is as shown in ~~the figure~~Figure 1 and ~~the table~~Table 1.

[0084] ~~The figure~~Figure 1 shows that the compound wang 520 in a final concentration of 30μM ~~have~~ has the best relative activity (94%) which is improved greatly compared with ~~the activity~~that of the compound 2f. In addition, the compounds as shown in ~~the table~~Table 1 have the dose dependency on the activity of GLP-1R, wherein the ~~median effective dose (~~EC_{50}~~)~~ of the compounds of wang-520, wang-516. wang-554, wang-488, wang516-2, wang502 and wang-504 ~~is~~ are less than 10μM. Such result provides the direction for determining the superior structure of the interaction of the compounds with GLP-1R.

[0085] ~~The table~~ Table1

~~The number of the compound~~ID	EC_{50}/μM
wang524	46.5
wang520	4.6
wang462	11.6
wang516	6.85
2f	13.0
wang866	54.41
wang554	5.24

wang488	6.73
wang516-2	6.06
wang502	3.31
wang504	4.87

[0086] 2. Determination of the concentration of intracellular cAMP

[0087] Since the determination of the concentration of intracellular cAMP indirectly by detecting the expression of the report gene is an indirect process, the functional re-screen is directly performed with the cAMP-detecting kit in order to make sure that the compound can surely increase the concentration of intracellular cAMP.

[0088] 2.1 Experimental material and instruments

[0089] cAMP-detecting kit (Applied Biosystems Cooperation)

[0090] Forma carbon dioxide incubator (Forma Cooperation)

[0091] Victor 2 according machine (Wallac Cooperation)

[0092] HEK 293/GLP1R+Luc strain which stably express GLP-1R and luciferase (National new medicaments screening center)

[0093] Candidate compound: the compound 2f

[0094] Standard cAMP (provided in the kit, Applied Biosystems Cooperation)

[0095] 2.2 Experimental process

[0096] HEK 293 cells are inoculated into 96-well plate in 20000 cells/100μl/well, which is ~~are~~ cultured at ~~37~~ 37° overnight. The compound 2f is diluted to 1.00E-03M, 1.00E-04M, 1.00E-05M, 1.00E-06M and 1.00E-07M with dimethyl sulphoxide, ~~following~~ followed by being inoculated into the above 96-well plate in 1l/well and being cultured at 37 with 5% of CO_2 for 1 hour. The concentration of intracellular cAMP is

detected according to the specification of cAMP-Screen Direct TM system kit.

[0097] 2.3 Experimental result

[0098] The ~~determining~~ final result of the concentration of intracellular cAMP is shown in ~~the figure~~Figure 2. As shown in ~~the figure~~ Figure 2, with the increase of the concentration of the compound 2f, the concentration of cAMP which is produced under this stimulation shows an exponential increase. This indicates that the compound 2f has a certain effect on signal transmission of GLP-1R as a GLP-1R agonist. When the concentration of the compound 2f increases to 30μM and 100μM, the concentration of cAMP shows the decreasing trend, which is caused by the cellulotoxic effect of the high concentration of the compound 2f.

[0099] 3. The test on the ligand-binding activity

[0100] In order to determine the binding ~~activity~~ activities of the ~~compound to the ligand~~compounds, the cells which highly express GLP-1R are prepared, GLP-1 labelled with ^{125}I is used as the ligand, while adding into the candidate compound. When the candidate compound binds the ^{125}I-labelled GLP-1 competitively, the isotope labels on the cell membrane reduce. Accordingly, the affinity of the candidate compound to the ligand can be evaluated (J Mol Endocrinol. 2000 Vol.25:321-35; J Biomol Screen, 2000 Vol. 5:377-84).

[0101] 3.1 Experimental material and instruments

[0102] HEK 293/GLP1R+Luc cell strain (National New Medicaments Screening Center)

[0103] Labeled compound: ^{125}I-labelled GLP-1 (Amersham Biosciences Cooperation)

[0104] Wallac MicroBata work station (Perkin Elmer Cooperation)

[0105] TomTech cell collector (TomTec Cooperation)

[0106] The testing buffer solution:

[0107] 20mM of tris-HCl (pH 7.4) (Shanghai Shenggong biological engineering technology LTD), 100mM of NaCl (Shanghai Chemical agents Cooperation), 15mM of NaF (Shanghai Chemical agents Cooperation), 2mM of deoxypyridoxine (Sigma Cooperation), 0.2mM of phenylmethylsulfonyl fluoride (Sigma Cooperation), aprotinin (Shanghai Chemical agents Cooperation) (1μg/ml), and –leupeptin (Shanghai Chemical agents Cooperation) (1μg/ml).

[0108] The washing solution:

[0109] 20mM of tris-HCl (pH 7.4), 100mM of NaCl, and 15mM NaF

[0110] The scintillation liquid (Wallac Cooperation)

[0111] The candidate compound is diluted with dimethyl sulphoxide at the concentration gradient of 0.1nM, 1nM, 10nM, 100nM, 1000nM, 10,000nM, and 100,000 nM.

[0112] 3.2 Experimental process

[0113] 10^5 HEK 293/GLP1R+Luc cells in the logarithmic growth phase are incubated together with the ^{125}I-labelled GLP-1 positive peptide (the final concentration of 40pM) in 200μl of the testing buffer solution at 25 for 4 hours, while adding into the non-labeled positive peptide or the candidate compound. The cells are collected with the cell collector, ~~following~~ followed by washing three times with the washing solution. The scintillation liquid is added into them, and each well is counted with Microbata counter.

[0114] 3.3 Experimental result

[0115] The result of the receptor-binding experiment is shown in ~~the table~~Table 3. As shown in ~~the table~~Table 3, the compound 2f has the better affinity to GLP-1R, but the affinities of compounds wang520 and wang516 are little ~~week~~weak, and ~~the~~ other

~~compound~~ compounds substantially don't bind to the receptor in the testing concentration ~~rage~~range.

[0116] ~~The table~~Table 3

~~The number of the compound~~ID	EC_{50}/μM
wang524	>100μM
wang450	>100μM
wang405	>100μM
wang327	>100μM
wang520	60-100μM
wang462	>100μM
wang866	>100μM
wang516	40-80μM
wang420	>100μM
2f	31μM